LESSON 3:
CULTURE MEDIA

Objective

When cultured in vitro, all the needs, both chemical and physical, of the plant cells have to be met by the culture vessel, the growth medium and the external environment (light, temperature, etc.). The growth medium has to supply all the essential mineral ions required for growth and development. In many cases (as the biosynthetic capability of cells cultured in vitro may not replicate that of the parent plant), it must also supply additional organic supplements such as amino acids and vitamins. Many plant cell cultures, as they are not photosynthetic, also require the addition of a fixed carbon source in the form of a sugar (most often sucrose). One other vital component that must also be supplied is water, the principal biological solvent. Physical factors, such as temperature, pH, the gaseous environment, light (quality and duration) and osmotic pressure, also have to be maintained within acceptable limits.

Now, you must have understood that growth and morphogenesis of plant tissue in vitro largely depend on the composition of the culture media. No single medium can be suggested for all types of plants and organs. So, the details of culture medium have to be worked out for each plant material separately. Several media have been developed which are commonly used e.g.,

i. Murashige & Skoog's medium (MS medium)

ii. Gamborg et al. (B5 medium).

iii. White's medium.

The most important of these media is MS medium.

Media Composition

The major constituents of most plant tissue culture media are:

i. Inorganic nutrients (macro - and micro-nutrients)

ii. Carbon source (sugar)

iii. Organic supplements

iv. Growth regulators

v. A gelling / solidifying agent (agar).

The concentration of inorganic and organic constituents in culture media is expressed either in mass value (mg/l or ppm) or mole values (1M or 1 mol/l).

Let us now discuss each constituent of the media in detail.

Inorganic Nutrients

A variety of mineral elements (salts) supply the needed macro - and micronutrients required in the life of a plant. Elements required in concentrations greater than 0.5 m mol L^{-1} are referred to as Macronutrients and those less than 0.05 mM/L as Micronutrients.

Macronutrients

They include six major elements:

Nitrogen(N), Phosphorus(P), Potassium(K), Calcium (Ca), Magnesium (Mg) and Sulphur (S), present as salts in the media. All are essential for plant cell & tissue growth. Culture media should contain at least 25 mmolL^{-1} nitrate and potassium. Better results are obtained if the source of 'N' in media is contributed by both nitrates and ammonium (2-20mmol L^{-1}) or any other reduced nitrogen source. Other major elements Ca, P, S and Mg, at concentrations in the range of 1-3 mmolL^{-1} appear adequate (see Table 1). The physiological role of various elements is summarized in Table 2.

Micronutrients:

These are Iron (Fe), Manganese (Mn), Zinc (Zn), Boron(B), Copper (Cu) and Molybdenum (Mo). Chelated forms of Fe and Zn are commonly used in preparing culture media eg. for embryo induction, EDTA-iron chelate is used in place of iron citrate. Generally, 0.1 u mol L^{-1} Cu and Co, 1 umol L^{-1} Fe and Mo, 5 u mol L^{-1} I, 5- 30u mol L^{-1} Zn, 20- 90 umol L^{-1} Mn and 2-5100 u mol L^{-1} B are added to culture media, depending upon the requirement of the experiment (see Table 1).

TABLE 1. Compositions of some plant tissue culture media.

Compound	Concentration (mg/l)		
	White's[1]	MS[2]	B5[3]
Inorganic			
NH_4NO_3	–	1650	–
KNO_3	80	1900	2527.5
$MgSO_4.7H_2O$	750	370	246.5
KH_2PO_4	–	170	–
$(NH_4)_2SO_4$	–	–	134
$Ca(NO_3)_2.4H_2O$	300	–	–
Na_2SO_4	200	–	–
$NaH_2PO_4.H_2O$	19	–	150
KCI	65	–	–
$CaCl_2.2H_2O$	–	440	150
Kl	0.75	0.83	0.75
H_3BO_3	1.5	6.2	3
$MnSO_4.4H_2O$	5	22.3	–
$MnSO_4.H20$	–	–	10
$ZnSO_4.7H_2O$	3	8.6	2
$Na_2MoO_4.2H_2O$	–	0.25	0.25
MoO_3	0.001	–	–
$CuSO_4.5H_2O$	0.01	0.025	0.025
$CoCl_2.6H_2O$	–	0.025	0.025
$Fe_2(SO_4)_3$	2.5	–	–
$FeSO_4.7H_2O$	–	27.8	–
$Na_2.EDTA.2H_2O$	–	37.3	–

Sequestrene 330 Fe[4]	–	–	28
Organic			
(a) Vitamins			
Inositol	–	100	100
Nicotinic acid	0.05	0.5	1
Pyridoxine HCI	0.01	0.5	1
Thiamine HCI	0.01	0.1	10
(b) Amino acid			
Glycine	3	2	–
(c) Carbon source			
Sucrose (g/l)	20	30	20
(d) Growth regulators	As per need(Section 3.5.4)		

Gelling agent (for callus cultures)

Agar Generally 6-8 g/1 of the medium

Medium pH generally adjusted to5.8 with

IN HCI or IN NaOH

1. White, P.R. 1963. The Cultivation of Animal and Plant Cells. The Ronald Press, New York.
2. Murashige, T. and/Skoog, F. 1962. Physiol. Plant. 15: 473-497.
3. Gamburg, O.L., Miller, R.A. and Ojima, K. 1968. Exp. Cell Res. 50 : 151-158.

4. Fe -Na EDTA may be used when this compound is not available.

TABLE 2. Some of the elements important for plant nutrition and their physiological function.

These elements have to supplied by the culture medium in order to support the growth of culture.

Element	Function
Nitrogen	Component of proteins, nucleic acids and some coenzymes element required in greatest amount
Potassium	Regulates osmotic potential, principal inorganic cation
Calcium	Cell wall synthesis, membrane function, cell signalling
Magnesium	Enzyme cofactor, component of chlorophyll
Phosphorus	Component of nucleic acids, energy transfer, component of intermediates in respiration and photosynthesis
Sulphur	Component of some amino acids (methionine, cysteine) and some cofactors
Chlorine	Required for photosynthesis
Iron	Electron transfer as a component of cytochromes
Manganese	Enzyme cofactor
Cobalt	Component of some vitamins
Copper	Enzyme cofactor, electron-transfer reactions
Zinc	Enzyme cofactor, chlorophyll biosynthesis
Molybdenum	Enzyme cofactor, component of nitrate reductase
Nitrogen	is most commonly supplied as a mixture of nitrate ions (from the KNO_3) and ammonium ions (from the NH_4NO_3).

Theoretically, there is an advantage in supplying nitrogen in the form of ammonium ions, as nitrogen must be in the reduced form to be incorporated into macromolecules. Nitrate ions therefore need to be reduced before incorporation. However, at high concentrations, ammonium ions can be toxic to plant cell cultures and uptake of ammonium ions from the medium causes acidification of the medium. In order to use ammonium ions as the sole nitrogen source, the medium needs to be buffered. High concentrations of ammonium ions can also cause culture problems by increasing the frequency of vitrification (the culture appears pale and 'glassy' and is usually unsuitable for further culture). Using a mixture of nitrate and ammonium ions has the advantage of weakly buffering the medium as the uptake of nitrate ions causes OH– ions to be excreted. Phosphorus is usually supplied as the phosphate ion of ammonium, sodium or potassium salts. High concentrations of phosphate can lead to the precipitation of medium elements as insoluble phosphates.

Carbon and Energy Source

The most preferred carbon source in plant tissue culture is 'Sucrose'. Glucose supports equally good growth, while fructose is less efficient. Sucrose, while autoclaving the medium is converted to glucose and fructose. In the process, first glucose is used and then, fructose.

Plant cells and tissues in the culture medium lack autotrophic ability and therefore, need external carbon for energy. Even tissues which are initially green or acquire green pigments under special conditions during the culture period are not autotrophs for carbon. The addition of an external carbon source to the medium enhances proliferation of cells and regeneration of green shoots.

Organic Supplements

1. Vitamins: Plants synthesise vitamins endogenously and these are used as catalysts in various metabolic processes. When plant cells and tissues are grown in vitro, some essential vitamins are synthesized but only in sub- optimal quantities. Hence, it is necessary to supplement the medium with required vitamins and amino acids to achieve the best growth of the tissue. Thiamine (B1), nicotinic acid (B3), pyridoxine (B6), calcium pantothenate (B5) and myo inositol are used more often, in the range of 0.1- 10.0 mg/l.
2. Amino Acids: Cultured tissues are normally capable of synthesizing the amino acids necessary for various metabolic processes. In spite of this, the addition of amino acids to the media is important for stimulating cell growth in protoplast cultures and for establishing cell lines. Casein

hydrolysate (0.05- 0.1%), L- glutamine (8 mmol/l), L-asparagines (100 mmol/l), L- glycine (2mmolL^{-1}), L-arginine and L-cysteine (10mmolL^{-1}) are common sources of organic nitrogen used in culture media.

3. Other Organic Supplements: These include organic extracts eg. protein (Casein) hydrolysates, coconut milk, yeast and malt extracts, ground banana, orange juice and tomato juice. They are of undefined nature. In tissue culture, success achieved with coconut milk (5 to 20%) and protein (casein) hydrolysate (0.05 to 1.0%) has been significant. Potato extract has been found a suitable medium for anther culture.
4. Activated Charcoal: The addition of activated charcoal (AC) to culture media stimulates growth and differentiation in orchids, carrot, ivy and tomato. AC adsorbs inhibitory compounds and darkening of the medium occurs. AC is generally acid-washed and neutralised before its addition at concentrations of 0.5- 3% to the culture medium. It also helps to reduce toxicity by removing toxic compounds (eg. Phenols) produced during the culture and permits unhindered cell growth.
5. Antibiotics: Some plant calls have a systemic infection of microorganisms. To prevent the growth of these microbes, it is essential to enrich the media with antibiotics, eg. Streptomycin or kanamycin at low concentration effectively controls systemic infection and do not inhibit the growth of cell cultures.

Growth Regulators

These include: i) auxins, ii) cytokinins, iii) Gibberellins, iv) Abscisic acid and, (v) Ethylene. The growth, differentiation and organogenesis of tissues occurs only on the addition of one or more of these hormones to the medium.

Auxins

Commonly used auxins are : (Table 3)

i. Indole-3 - acetic acid (IAA).

ii. Indole –3- butyric acid (IBA)

iii. 2,4-dichlorophenoxyacetic acid (2,4-D)

iv. Naphthoxyacetic acid (NOA)

IAA occurs naturally in the plant tissues. Auxins have the property of inducing cell division. In nature, hormones of this group are involved in elongation of stem, internodes, tropism, apical dominance, abscission and rooting. 2, 4-D is mostly employed to induce callus production. Auxins are generally dissolved either in ethanol or dilute NaOH.

Cytokinins

These are adenine derivatives which are mainly concerned with cell division, modification of apical dominance and shoot differentiation, in the tissue culture. Commonly used cytokinins include: (Table 3)

i. 6-Benzylaminopurine (BAP)

ii. Isopentyl-adenine (2,ip)

iii. Furfurylamino purine (Kinetin).

iv. Zeatin.

v. 6- Benzyladenine (BA).

Zeatin and 2,ip are naturally occurring cytokinins. Cytokinins are generally dissolved in dilute HCL or NaOH.

The ratio of auxins and cytokinins is important with respect to morphogenesis in the culture system. For embryogenesis, callus initiation and root initiation, the ratio of auxins to cytokinin is high, while the reverse leads to axillary and shoot proliferation. Equally important is concentration of the two hormones. Eg. 2,4-D and BA at a concentration of 5.0 mgL^{-1} promote callus formation, but if used at 0.1 mg L^{-1} concentration, they promote shoot formation. Cytokinins have been shown to activate RNA synthesis and to stimulate protein and enzyme activity in certain tissues.

Table 3. Commonly used Auxins, their abbreviations and Chemical name

Abbreviation/name	Chemical name
2,4-D	2,4-dichlorophenoxyaceti
2,4,5-T	2,4,5-trichlorophenoxyac
Dicamba	2-methoxy-3,6-dichlorobe
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
MCPA	2-methyl-4-chlorophenox
NAA	1-naphthylacetic acid
NOA	2-naphthyloxyacetic acid
Picloram	4-amino-2,5,6-trichloropi

Commonly used cytokinins, their abbreviation and chemical name

Abbreviation/name	Chemical name
BAP	6-benzylaminopurine
2iP (IPA)	(2-isopentyl)adenine
Kinetin	6-furfurylaminopurine
Thidiazuron	1-phenyl-3-(1,2,3-thiadiazol-5-yl)urea
Zeatin	4-hydroxy-3-methyl-trans-2-butenylaminopurine

Gibberellins and Abscisic acid: GA_3 is the most common gibberellin used. It promotes the growth of cell cultures at low density, enhances callus growth and induces dwarf or stunted plantlets to elongate. GA_3 is used to induce plantlets formation from adventive embryos formed in culture.

Abscisic acid (ABA) in the culture medium either stimulates or inhibits the callus growth depending on the species.

Ethylene

Ethylene is a gaseous, naturally occurring, plant growth regulator most commonly associated with controlling fruit ripening in climacteric fruits, and its use in plant tissue culture is not widespread. It does, though, present a particular problem for plant tissue culture. Some plant cell cultures produce ethylene, which, if it builds up sufficiently, can inhibit the growth and development of the culture. The type of culture vessel used and its means of closure affect the gaseous exchange between the culture vessel and the outside atmosphere and thus the levels of ethylene present in the culture.

Solidifying Agents

Gelling or solidifying agents are commonly used for preparing semi-solid or solid tissue culture media. Agar (a polysaccharide obtained from seaweeds) is used to provide solid surface for growth because in the liquid medium, the tissue will be submerged and die due to lack of available oxygen. Agar is not a nutrient. Cells can also be grown in suspension cultures devoid of agar, but such cultures need to be aerated regularly either by bubbling sterile air or by gentle agitation.

Agar has several advantages over other gelling agents:

i. Agar does not react with media constituents

ii. It is not digested by plant enzymes and remains stable at all possible incubation temperatures.

Normally, 0.5 to 1% agar is used in the medium to form a firm gel at the pH typical of plant culture media.

pH:

Plant cells and tissues require optimum pH for growth and development in cultures. The pH affects uptake of ions and for most of the culture media pH 5.0 to 6.0 before sterilization is considered optimal. Higher pH is likely to give a hard medium while a low pH results in unsatisfactory solidification of agar. While preparing a medium, the pH can be adjusted to the requirement of an experiment.

Media Preparation:

It can be prepared as follows:

i. Now a days, plant tissue culture media most commonly used are available in the market as dry powders. So, the simplest method is to dissolve these powders containing inorganic and organic nutrients, in distilled water. After mixing thoroughly, sugar and agar (melted) and other organic supplements are added. Finally, the volume is made up to 1 litre, pH is adjusted and medium is then autoclaved (See Appendix 1.).

ii. For experiments where changes in the quantity and quality of media constituents becomes necessary, it is desirable to weigh and dissolve each ingredient separately before mixing them together.

iii. In this, 4 stock solutions are prepared, consisting of i) major salts (20 x concentrated) ii) minor salts (200 x concentrated); iii) iron (200x concentrated) and iv) organic nutrients except sucrose (200 x concentrated) (see Appendix 2.). For each growth regulator, a separate stock solution is prepared. All the stock solutions are stored in proper plastic or glass containers at low temperature. Iron stock is stored in colored bottles. Extra care is needed for storing coconut milk. The liquid extract (endosperm,) of the fruit is boiled to deproteinise it, filtered and stored in plastic bottles in a deep-freeze at –20°C. Contaminated or precipitated solution should not be used (See Appendix 3.). Banana powder is also used in certain media preparations (see Appendix 3.).

Appendix 1 : Preparation from Packaged Powder

Powdered media are extremely hygroscopic and must be protected from atmospheric moisture. If possible the entire contents of each package should be used immediately after opening. Preparing the medium in a concentrated form is not recommended as some salt complexes may precipitate. Supplements that are added to the medium may affect shelf life and storage conditions. The basic steps for preparing the culture medium are listed below:

1. Measure out approximately 90% of the final required volume of tissue culture grade water, e.g. 900 ml for a final volume of 1000 ml. Select a container twice the size of the final volume.
2. While stirring the water add the powdered medium and stir until completely dissolved. Heating may be required to bring powders into solution.
3. Rinse the original container with a small volume of tissue culture grade water to remove traces of the powder. Add to the solution in Step 2.
4. Add desired heat stable supplements (e.g. sucrose, gelling agent, vitamins, auxins, cytokinins, etc.)
5. Add additional tissue culture grade water to bring the medium to the final volume.
6. While stirring, adjust medium to desired pH using NaOH, HCl or KOH.
7. If a gelling agent is used, heat until the solution is clear.
8. Dispense the medium into the culture vessels before (or after) autoclaving according to your application. Add heat labile constituents after autoclaving.
9. Sterilize the medium in a validated autoclave at 1 kg/cm2 (15 psi), 121 °C, for the time period described under Sterilization of Media Protocol.
10. Allow medium to cool prior to use.

Powdered media and basal salt mixtures are for laboratory use only. Not for drug, household or other uses.

Storage

Store dry medium in a desiccator at 0-5 °C. Deterioration of powdered medium may be recognized by: 1) color change; 2) granulation, clumping, or particulate matter throughout the powder; 3) insolubility; 4) pH change; or 5) inability to promote growth when properly used.

Precipitation in Media

Precipitates are known to occur, with time, in plant tissue culture media. The precipitates have been analyzed (Sigma, unpublished data; Dalton, et al. 1983). They are composed of small, pale yellow-white particles. Analysis of precipitates indicated a predominance of iron, phosphate, and zinc. The probable cause of the precipitates is the inevitable oxidation of ferrous ions to ferric ions and the presence of unchelated ferric ions. When the solubility of ferric phosphate is exceeded precipitation occurs. There are no reports of detrimental effects on growth and development in plant tissue culture due to the precipitates.

Appendix 2 : Preparation from Basal Salt Solutions

Liquid 20X solutions are offered for your convenience. To avoid precipitation over long term storage, Sigma has formulated two solutions which when mixed at the proper dilution make a

solution with the appropriate salt concentration. The basic steps for preparing 1 liter of culture medium are listed below. CAUTION: Do not autoclave product in bottle. The bottle is NOT autoclavable.

1. Measure approximately 700 ml of tissue culture grade water .
2. While stirring the water, add 50 ml of Macronutrient Solution.
3. Continue stirring the mixture while adding 5 ml of Micronutrient Solution.
4. Add desired heat stable supplements (e.g. sucrose, gelling agent, vitamins, auxins, cytokinins, etc.).
5. Add additional tissue culture grade water to bring the medium to the final volume.
6. While stirring, adjust medium to desired pH using NaOH, HCl or KOH.
7. If a gelling agent is used, heat until the solution is clear.
8. Dispense the medium into the culture vessels before (or after) autoclaving according to your application. Add heat labile constituents after autoclaving.
9. Sterilize the medium in a validated autoclave at 1 kg/cm2 (15 psi), 121 °C, for the time period described under Sterilization of Media Protocol.
10. Allow medium to cool prior to use.

Storage

Store basal salt solutions at 0-5 °C. Deterioration of basal salt solutions may be recognized by: 1) color change; 2) pH change; 3) precipitation of components; or 4) inability to promote growth when properly used.

[* No filter found for the requested operation. | In-line *]

Appendix 3 : Banana Powder

Sigma offers a banana powder for use in orchid and other plant cell culture. Product number B4032 is a powder from a spray-dried mixture of banana and dextrin. Use this product at approximately 40 g/L. To reduce clumping, add powder slowly to the culture medium with constant stirring. The presence of banana solids is common in medium containing both of these products.

Coconut Water

Coconut water has been shown to stimulate shoot proliferation in many species of plants. Coconut water is prepared from selected coconuts and processed to remove most of the protein. The product is then filter sterilized and frozen prior to shipment. Remaining protein levels in the water may vary from one lot to the next and may result in precipitate when the product is frozen. This precipitation should not effect the growth of the plant tissue. The precipitate can be removed by filtering or by allowing it to settle to the bottom of the bottle and then decanting. Coconut water can be divided into smaller aliquots, corresponding to your standard medium batch size, and refrozen until needed. Coconut water should be used at a concentration of 5-20% (v/v).

Conclusion

Although the basic requirements of cultured plant tissues are similar to those of whole plants, in practice, nutritional components promoting optimal growth of a tissue under laboratory conditions may vary with respect to the particular species. Media compositions are therefore, formulated considering specific requirements of a particular culture system. Considerable progress has been made during the past decades on the development of media for growing plant cells, tissues, and organs aseptically in culture. It is therefore, concluded that culture media has to be designed in such a way that it not only influences the growth of cells or explants but also helps further in achieving the regeneration of plants.

Questions

1. How will you prepare a culture medium? Write the formulation and nutritional components of any one culture medium.
2. Give a brief account of the growth regulators used in the medium.

Appendix 4 : Plant Tissue Culture in Your Kitchen?

- How does all of this translate into something the hobbyist can use?

  Plant tissue culture can be done in the kitchen, using a microwave oven to sterilize media, PPM, a biocide, to minimize contamination, and a simple box to limit dust falling into culture vessels .
  Premixed plant media can be purchased, but with some experimentation, homemade media might suffice for your particular plant. See recipe below. Some chemicals are difficult to find even at the garden stores, such as the cytokinins benzylaminopurine and isopentyladenine, but these can be purchased in small, inexpensive quantities. You may find that your particular plant does not require them!

Most supplies needed for home tissue culture can be found in your kitchen: bleach, detergent, vinegar, sugar, plate, knife,etc.

A simple plastic box will serve as a clean area. Pint jars can be used to hold alcohol, bleach, and sterile water for disinfecting plants.

- What do I use for culture vessels instead of test tubes and flasks?

  Baby food jars make great culture vessels. They can be sterilized in a pressure cooker with the original metal caps or in a microwave with polypropylene caps. Various food containers, such as Gladware, can also be used as culture vessels and processed in the microwave. If you are not sure how a particular plastic will hold up in the microwave, be sure to do a test run and have a paper plate under the item.

- Where do I get distilled water? How do I sterilize it?

  Tap water, or filtered water, such as Brita, can be used. Water can be microwaved for 10 minutes or processed in the pressure cooker for 20-30 minutes.

- How do I make plant medium? How do I know which medium to use for my particular species?

  Directions for making homemade medium can be found below. Determining which medium to use will require some research: surf the net, ask others on the "home tissue culture listserv", or go to the library.

- How do I sterilize my forceps and knife? What do I use for a sterile cutting surface?

  In the university labs, we prefer using ethanol for sterilizing instruments. It smells better than isopropanol and seems to be gentler on your hands and on the plants. In our home labs, isopropanol, purchased at WalMart or Kmart will do just fine. Forceps and knives should be dipped in the isopropanol, shaken to rid of excess liquid, and then used to cut the sterilized plant material. A small salad plant can be wiped off with isopropanol and will serve as a sterile cutting surface.

- Where do I put my cultures once that are started?

  Michael Kane's papers recommend maintaining the cultures at about 25 C under a 16 hour photoperiod provided by cool-white fluorescent tubes. Basically that translates to: room temperature, shop lights from Kmart or WalMart, and metal shelves from which to hang the light units. Lights should be about 9-12 inches from the baby food jars. Never put your cultures on the window sill or in direct sunlight. The temperature extremes will be too great.

Media Recipes (Kitchen Style)

Ingredients for Basal MS Medium

MS medium package 2 tablespoons table sugar 1 ml PPM Agar tap water or distilled water vinegar and antacid tablet to adjust pH Optional hormones

Ingredients for "Homemade" Medium" 1 teaspoon Peter's fertilizer (20-20-20) 2 tablespoon sugar 1 multivitamin pill (do not crush - allow to partially dissolve only and then remove after a few minutes) 1 ml PPM Agar tap water or distilled water vinegar and antacid tablet to adjust pH Optional hormones

Instructions for Media Preparation:

Fill quart jar with about 3 cups water Add MS medium (or Peter's fertilizer) and 2 tablespoons table sugar and mix well While wearing gloves and goggles, add pre-measured PPM and any necessary hormones to the quart jar; mix well. Bring volume to 1 quart with more water. Test the pH of the solution by dipping the edge of a piece of pH paper into the solution. A pH of 5.5 to 6.0 is preferred. Compare the color of the wet pH paper to the pH color chart:If the pH is too low ("acidic"), add a pinch of baking soda. Mix well and test the pH again. If the pH is too high ("basic"), add a few milliliters of vinegar. Stir to mix and test the pH again. Continue this process until the pH is between 5.5 and 6.0.Add 3 tablespoons of liquid medium to each baby food jar using a plastic measuring tablespoon. Add 1/16 teaspoon PhytoTechnology Lab's Agar, or one level "pink Baskin-Robbins" spoon to each baby food jar. Place the polypropylene baby food jar caps on the jars and press to tighten. These can be processed in the microwave for about 3 minutes (see Stiff, 1998) or in a pressure cooker for 30 minutes. Metal baby food jar caps can be used in a pressure cooker.

Cleaning up Plant Material

While concentrations of bleach used and the duration of soaking time in the bleach will vary from plant to plant, the basic methodology is the same.

>Bleach solutions are made in the following way. Safety clothing and equipment are recommended (gloves, glasses, apron, old clothers, shoes, and a respirator if you have a chlorine allergy)

Note that all commercial bleach is not the same. The common commercial bleach in the States is 5.25% sodium hypochlorite. This concentration will vary in other countries so be aware of the concentration of the solution you are using. The following is based on used U.S. bleach:

10% bleach	=	1/4 cup bleach + 2 1/4 cup water + few drops detergent
25% bleach	=	1/4 cup bleach + 3/4 cup water + few drops detergent
50% bleach	=	1/2 cup bleach + 1/2 cup water + few drops detergent

The following website(s) show the basics of how to clean up plant material for tissue culture: www.kitchenculturekit.com/africanviolet.htm

References of Interest

Ailstock, M.S., W.J. Fleming, and T.J. Cooke. 1991. The characterization of axenic culture systems suitable for plant propagation and experimental studies of the submerged aquatic angiosperm Potamogeton pectinatus (sago pondweed). Estuaries 14: 57-64.

Bird, K.T. 1993. Salinity effects on Ruppia maritima L. cultured in vitro. Botanica Marina 36:23-28.

Bird, K.T. and J.Jewett-Smith. 1994. Development

Note